

In the Specification:

At page 1, line 7, insert

Cross Reference to Related Applications

This application is a continuation of, and claims priority from, United States patent application 09/867,852 filed May 29, 2001 which is a continuation of U.S. Serial No. 09/301,085, filed on April 28, 1999, which is a divisional of U.S. Serial No. 08/310,912, filed September 22, 1994, which, in turn, is a continuation-in-part of U.S. patent application 08/227,360, filed April 13, 1994.

Delete the current Sequence Listing located at the end of the application and replace it with the new Sequence Listing submitted herewith.

Replace the paragraph beginning on page 4, line 29, with the following paragraph.

In other aspects, the invention features a substantially pure oligonucleotide including one or a combination of the sequences:

5' GGNATGGGNGGNNTNGGNAARACNAC 3' (SEQ ID NO:158), wherein N is A, T, G, or C; and R is A or G;

5' NARNGGNARNCC 3' (SEQ ID NO:169), wherein N is A, T, G or C; and R is A or G;

5' NCGNGWNGTNAKDAWNCGNGA 3' (SEQ ID NO:159), wherein N is A, T, G or C; W is A or T; D is A, G, or T; and K is G or T;

5' GGWNTBGGWAARACHAC 3' (SEQ ID NO:160), wherein N is A, T, G or C; R is G or A; B is C, G, or T; H is A, C, or T; and W is A or T;

5' TYGAYGAYRTBKRBRA 3' (SEQ ID NO:163), wherein R is G or A; B is C, G, or T; D is A, G, or T; Y is T or C; and K is G or T;

5' TYCCAVAYRTCRTCNA 3' (SEQ ID NO:164), wherein N is A, T, G or C; R is G or A; V is G or C or A; and Y is T or C;

5' GGWYTBCCWYTBGCHYT 3' (SEQ ID NO:170), wherein B is C, G, or T; H is A, C, or T; W is A or T; and Y is T or C;

5' ARDGCVARWGGVARNCC 3' (SEQ ID NO:171), wherein N is A, T, G or C; R is G or A; W is A or T; D is A, G, or T; and V is G, C, or A; and

5' ARRTTRTCRTADSWRAWYTT 3' (SEQ ID NO:174), wherein R is G or A; W is A or T; D is A, G, or T; S is G or C; and Y is C or T.

Replace the paragraph beginning on page 5, line 19, with the following paragraph.

In other aspects, the invention features a recombinant plant gene including one or a combination of the DNA sequences:

5' GGNATGGGNGGNNTNGGNAARACNAC 3' (SEQ ID NO:158), wherein N is A, T, G or C; and R is A or G;

5' NARNGGNARNCC 3' (SEQ ID NO:169), wherein N is A, T, G or C; and R is A or G;

5' NCGNGWNGTNAKDAWNCGNGA 3' (SEQ ID NO:167), wherein N is A, T, G or C; W is A or T; D is A, G or T; and K is G or T.

Replace the paragraph beginning on page 5, line 28, with the following paragraph.

In another aspect, the invention features a substantially pure plant polypeptide including one or a combination of the amino acid sequences:

Gly Xaa<sub>1</sub> Xaa<sub>2</sub> Gly Xaa<sub>3</sub> Gly Lys Thr Thr Xaa<sub>4</sub> Xaa<sub>5</sub> (SEQ ID NO:191),

wherein Xaa<sub>1</sub> is Met or Pro; Xaa<sub>2</sub> is Gly or Pro; Xaa<sub>3</sub> is Ile, Leu, or Val; Xaa<sub>4</sub> is Ile, Leu, or Thr; and Xaa<sub>5</sub> is Ala or Met;

Xaa<sub>1</sub> Xaa<sub>2</sub> Xaa<sub>3</sub> Leu Xaa<sub>4</sub> Xaa<sub>5</sub> Xaa<sub>6</sub> Asp Asp Xaa<sub>7</sub> Xaa<sub>8</sub> (SEQ ID NO:192),

wherein Xaa<sub>1</sub> is Phe or Lys; Xaa<sub>2</sub> is Arg or Lys; Xaa<sub>3</sub> is Ile, Val, or Phe; Xaa<sub>4</sub> is Ile, Leu, or Val; Xaa<sub>5</sub> is Ile or Leu; Xaa<sub>6</sub> is Ile or Val; Xaa<sub>7</sub> is Ile, Leu, or Val; and Xaa<sub>8</sub> is Asp or Trp;

Xaa<sub>1</sub> Xaa<sub>2</sub> Xaa<sub>3</sub> Xaa<sub>4</sub> Xaa<sub>5</sub> Thr Xaa<sub>6</sub> Arg (SEQ ID NO:193),

wherein Xaa<sub>1</sub> is Ser or Cys; Xaa<sub>2</sub> is Arg or Lys; Xaa<sub>3</sub> is Phe, Ile, or Val; Xaa<sub>4</sub> is Ile, or Met; Xaa<sub>5</sub> is Ile, Leu, or Phe; Xaa<sub>6</sub> is Ser, Cys, or Thr;

Gly Leu Pro Leu Xaa<sub>1</sub> Xaa<sub>2</sub> Xaa<sub>3</sub> Xaa<sub>4</sub> (SEQ ID NO:194),

wherein Xaa<sub>1</sub> is Thr, Ala, or Ser; Xaa<sub>2</sub> is Leu or Val; Xaa<sub>3</sub> is Ile, Val, or Lys; and Xaa<sub>4</sub> is Val or Thr; and

Xaa<sub>1</sub> Xaa<sub>2</sub> Ser Tyr Xaa<sub>3</sub> Xaa<sub>4</sub> Leu (SEQ ID NO:195),

wherein Xaa<sub>1</sub> is Lys or Gly; Xaa<sub>2</sub> is Ile or Phe; Xaa<sub>3</sub> is Asp or Lys; and Xaa<sub>4</sub> is Ala, Gly, or Asn.

Replace the paragraph beginning on page 17, line 6, with the following paragraph.

Fig. 2 is the complete nucleotide sequence of cDNA-4 comprising the *RPS2* gene locus (SEQ ID NO:1). The three reading frames are shown below the nucleotide sequence (SEQ ID NOS:2-104, 196, and 198-200). The deduced amino acid sequence of reading frame "a" is provided and contains 909 amino acids. The methionine encoded by the ATG start codon is circled in open reading frame "a" of Fig. 2 (SEQ ID NOS:2-5). The A of the ATG start codon is nucleotide 31 of Fig. 2 (SEQ ID NO:1).

Replace the paragraph beginning on page 17, line 13, with the following paragraph.

Fig. 3 is the nucleotide sequence of the *avrRpt2* gene (SEQ ID NO:105) and its deduced amino acid sequence (SEQ ID NO:106). A potential ribosome binding site is underlined. An inverted repeat is indicated by horizontal arrows at the 3' end of the open reading frame. The deduced amino acid sequence is provided below the nucleotide sequence of the open reading frame (SEQ ID NOS:105 and 106).

Replace the paragraph beginning on page 18, line 9, with the following paragraph.

Fig. 5A shows regions of sequence similarity between the L-6 protein of flax, N protein of tobacco, Prf protein of tomato, and rps2 protein of *Arabidopsis* (SEQ ID NOS:2, 107-136, 142, and 208).

Replace the paragraph beginning on page 18, line 12, with the following paragraph.

Fig. 5B shows sequence similarity between the N and L-6 proteins (SEQ ID NOS:107, 108, 129-136, 138-140, and 207).

Replace the paragraph beginning on page 18, line 14, with the following paragraph.

Fig. 6 shows a sequence analysis of RPS2 polypeptide showing polypeptide regions corresponding to an N-terminal hydrophobic region, a leucine zipper, NBSs (kinase-1a, kinase-2, and kinase-3 motifs), and a predicted membrane integrated region (SEQ ID NOS:141 and 142).

Replace the paragraph beginning on page 18, line 19, with the following paragraph.

Fig. 7 shows the amino acid sequence of the RPS2 LRR (amino acids 505-867) (SEQ ID NOS:143-156). The top line indicates the consensus sequences for the *RPS2* LRR (SEQ ID

NO:209). An "X" stands for an arbitrary amino acid sequence and an "a" stands for an aliphatic amino acid residue. The consensus sequence for the *RPS2* LRR is closely related to the consensus for the yeast adenylate cyclase CYR1 LRR (PX Xa XXL XXL XXLXL XXNXaXXa) (SEQ ID NO:210). The amino acid residues that match the consensus sequence are shown in bold. Although this figure shows 14 LRRs, the C-terminal boundary of the LRR is not very clear because the LRR closer to the C-terminus does not fit the consensus sequence very well.

Replace the paragraph beginning on page 18, line 31, with the following paragraph.

Fig. 8 shows a sequence analysis of *RPS2* (SEQ ID NO:142), indicating regions with similarity to leucine zipper, P-loop, membrane-spanning, and leucine-rich repeat motifs. Regions with similarity to defined functional domains are indicated with a line over the relevant amino acids. Potential N-glycosylation sequences are marked with a dot, and the location of the *rps2*-201 Thr to Pro mutation at amino acid 668 is marked with an asterisk.

Replace the paragraph beginning on page 20, line 7, with the following paragraph.

Fig. 12 shows a nucleic acid sequence of the tomato *Prf* gene (SEQ ID NO:157).

Replace the paragraph beginning on page 38, line 4, with the following paragraph.

As discussed above, we have discovered that the *Arabidopsis RPS2* gene described herein is representative of a new class of plant resistance genes. Analysis of the derived amino acid sequence for *RPS2* revealed several regions of similarity with known polypeptide motifs (see, e.g., Schneider et al., *Genes Dev.* 6:797 (1991)). Most prominent among these is a region of multiple, leucine-rich repeats (LRRs). The LRR motif has been implicated in protein-protein

interactions and ligand binding in a diverse array of proteins (see, e.g., Kornfield et al., Annu. Rev. Biochem. 64:631 (1985); Alber, Curr. Opin. Gen. Dev. 2:205 (1992); Lupas et al., Science 252:1162 (1991); Saraste et al., Trend Biochem. Sci. 15:430 (1990)). In one example, LRRs form the hormone binding sites of mammalian gonadotropin hormone receptors (see, e.g., Lupas et al., Science 252:1162 (1991)) and, in another example, a domain of yeast adenylate cyclase that interacts with the RAS2 protein (Kornfield et al., Annu. Rev. Biochem. 64:631 (1985)). In *RPS2*, the LRR domain spans amino acids 503-867 and contains fourteen repeat units of length 22-26 amino acids. A portion of each repeat resembles the LRR consensus sequence (I/L/V)XXLXXLXX(I/L)XL (SEQ ID NO:211). In Figure 7, the LRRs from *RPS2* are shown, as well as an *RPS2* consensus sequence. Within the *RPS2* LRR region, five (of six) sequences matching the N-glycosylation consensus sequence [NX(S/T)] were observed (Figure 8, marked with a dot). In particular, N-glycosylation is predicted to occur at amino acids 158, 543, 666, 757, 778, 787. Interestingly, the single nucleotide difference between functional *RPS2* and mutant allele *rps2-201* is within the LRR coding region, and this mutation disrupts one of the potential glycosylation sites.

Replace the paragraph beginning on page 39, line 4, with the following paragraph.

Also observed in the deduced amino acid sequence for *RPS2* is a second potential protein-protein interaction domain, a leucine zipper (see, e.g., von Heijne, J. Mol. Biol. 225:487 (1992)), at amino acids 30-57. This region contains four contiguous heptad repeats that match the leucine zipper consensus sequence (I/R)XDLXXX (SEQ ID NO:212). Leucine zippers facilitate the dimerization of transcription factors by formation of coiled-coil structures, but no sequences suggestive of an adjacent DNA binding domain (such as a strongly basic region or a



potential zinc-finger) were detected in *RPS2*. Coiled-coil regions also promote specific interactions between proteins that are not transcription factors (see, e.g., Ward et al., Plant Mol. Biol. 14:561 (1990); Ecker, Methods 1:186 (1990); Grill et al., Mol. Gen. Genet. 226:484 (1991)), and computer database similarity searches with the region spanning amino acids 30-57 of *RPS2* revealed highest similarity to the coiled-coil regions of numerous myosin and paramyosin proteins.

Replace the paragraph beginning on page 39, line 22, with the following paragraph.

A third *RPS2* motif was found at the sequence GPGGVGKT (SEQ ID NO:213) at deduced amino acids 182-189. This portion of *RPS2* precisely matches the generalized consensus for the phosphate-binding loop (P-loop) of numerous ATP- and GTP-binding proteins (see, e.g., Saraste et al., supra). The postulated *RPS2* P-loop is similar to those found in RAS proteins and ATP synthase  $\gamma$ -subunits (Saraste et al., supra), but surprisingly is most similar to the published P-loop sequences for the *nifH* and *chvD* genes, respectively. The presence of this P-loop sequence strongly suggests nucleotide triphosphate binding as one aspect of *RPS2* function. This domain is also referred to as a kinase-1a motif (or a nucleotide binding site, or NBS). Other conserved NBSs are present in the *RPS2* sequence; these NBSs include a kinase-2 motif at amino acids 258-262 and a kinase-3a motif at amino acids 330-335.

Replace the paragraph beginning on page 43, line 8, with the following paragraph.

Any number of probes and primers according to the invention may be designed based on the conserved RPS motifs described herein. Preferred motifs are boxed in the sequences shown

in Fig. 5(A or B). In particular, oligonucleotides according to the invention may be based on the conserved P-loop domain, the amino acids of which are shown below:

MOTIF 1

L6	G MGGIGKTTTA (SEQ ID NO:110)
N	G MGGVGKTTIA (SEQ ID NO:111)
PrfP	G MPGLGKTTLA (SEQ ID NO:112)
RPS2	G PGGVGKTTLM (SEQ ID NO:113)

From these sequences, appropriate oligonucleotides are designed and prepared using standard methods. Particular examples of RPS oligonucleotides based on the P-loop domain are as follows (N is A, C, T, or G).

Based on MOTIF 1:

5' GGNATGGGNGGNNTNGGNAA(A or G)ACNAC 3' (SEQ ID NO:158)

5' NCGNG(A/T)NGTNA(T/G)(G/A/T)A(T/A)NCGNA 3' (SEQ ID NO:159)

5' GG(T or A)NT(T or G or C)GG(T or A)AA(G or A)AC(T or C or A)AC 3' (SEQ ID NO:160)

5' GGNATGGGNGGNNTNGGNAA(A or G)ACNAC 3' (SEQ ID NO:158)



5' N(G or A)(C or T)N(A or G)(A or G or T)NGTNGT(C or T)TTNCCNANNCCN(G or C)(G or C)N(G or A)(T or G)NCC 3' (SEQ ID NO:161)

5' GGN(C or A)(T or C)N(G or C)(G or C)NGGNNTNGGNAA(A or G)ACNAC 3' (SEQ ID NO:162)

Replace the paragraph beginning on page 44, line 4, with the following paragraph.

Other conserved *RPS* motifs useful for oligonucleotide design are shown below. These motifs are also depicted in the sequence of Fig. 5(A or B).

MOTIF 2

L6	FKILVV LDDVD (SEQ ID NO:114)
N	KKVLIV LDDID (SEQ ID NO:115)
PrfP	KRFLIL IDDVW (SEQ ID NO:116)
RPS2	KRFLLL LDDVW (SEQ ID NO:117)

MOTIF 3

L6	SRFIIT SR (SEQ ID NO:118)
N	SRIIT TR (SEQ ID NO:119)
PrfP	SRIILT TR (SEQ ID NO:120)
RPS2	CKVMFT TR (SEQ ID NO:121)

MOTIF 4

L6	GLPLTLK V (SEQ ID NO:122)
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N GLPLALK V (SEQ ID NO:123)

PrfP GLPLSVV L (SEQ ID NO:124)

RPS2 GLPLALI T (SEQ ID NO:125)

MOTIF 5

L6 KISYDAL (SEQ ID NO:126)

N KISYDGL (SEQ ID NO:127)

PrfP GFSYKNL (SEQ ID NO:128)

RPS2 KFSYDNL (SEQ ID NO:208)

From the above motifs and the sequence motifs designated in Figure 5A and B, appropriate oligonucleotides are designed and prepared. Particular examples of such RPS oligonucleotides are as follows (N is A, T, C, or G).

Based on MOTIF 2:

5' T(T or C)GA(T or C)GA(T or C)(A or G)T(T or G or C)(T or G)(A or G)(T or G or C)(G or A)A 3' (SEQ ID NO:214)

5' T(T or C)CCA(G or C or A)A(T or C)(G or A)TC(A or G)TCNA 3' (SEQ ID NO:164)

5' (C or G or A)(T or C)(C or A)NA(T or C)(G or A)TC(G or A)TCNA(G or A or T)NA(G or A or C)NANNA(G or A)NA 3' (SEQ ID NO:165)

5' (T or A)(T or A)N(A or C)(A or G)(A or G)(T or G or A)TN(T or C)TNNTN(G or T or C)TN(A or T or C)TNGA(T or C)GA 3' (SEQ ID NO:166)

Based on MOTIF 3:

5' NCGNG(A or T)NGTNA(T or G)(G or A or T)A(T or A)NCGNGA 3' (SEQ ID NO:167)

5' NCGNG(A or T)NGTNA(T or G)(G or A or T)A(T or A)NCGNGA 3' (SEQ ID NO:167)

5' NC(G or T)N(G or C)(A or T)NGTNA(A or G or T)(A or G or T)AT(A or G or T)AATNG 3' (SEQ ID NO:168)

Based on MOTIF 4:

5' NA(G or A)NGGNA(G or A)NCC 3' (SEQ ID NO:169)

5' GG(T or A)(T or C)T(T or G or C)CC(T or A)(T or C)T(T or G or C)GC(T or C or A)(T or C)T 3' (SEQ ID NO:170)

5' A(A or G)(T or G or A)GC(G or C or A)A(G or A)(T or A)GG(G or C or A)A(G or A)(A or G or T or C)CC 3' (SEQ ID NO:171)

5' NA(G or A)NGGNA(G or A)NCC 3' (SEQ ID NO:169)

5' N(A or G)NN(T or A)(T or C)NA(G or C or A)N(C or G)(A or T or C)NA(G or A)NGGNA(G or A)NCC 3' (SEQ ID NO:172)

5' GGN(T or C)TNCCN(T or C)TN(G or A or T)(C or G)N(T or G or C)T 3' (SEQ ID NO:173)

Based on MOTIF 5:

5' A(A or G)(A or G)TT(A or G)TC(A or G)TA(G or A or T)(G or C)(T or A)(G or A)A(T or A)(C or T)TT 3' (SEQ ID NO:174)

5' A(G or A)N(T or C)(T or C)NT(C or T)(A or G)TAN(G or C)(A or G)NANN(C or T)(C or T) 3' (SEQ ID NO:175)

5' (G or A)(G or A)N(A or T)T(A or C or T)(T or A)(G or C)NTA(T or C)(G or A)AN(A or G)(A or C or G)N(T or C)T 3' (SEQ ID NO:176)

Based on MOTIF 6:

5' GTNTT(T or C)(T or C)TN(T or A)(G or C)NTT(T or C)(A or C)G(A or G)GG 3' (SEQ ID NO:177)

Based on MOTIF 7:

5' CCNAT(A or C or T)TT(T or C)TA(T or C)(G or A)(T or A)(G or T or C)GTNGA(T or C)CC 3' (SEQ ID NO:178)

Based on MOTIF 8:

5' GTNGGNAT(A or C or T)GA(T or C)(G or A)(A or C)NCA 3' (SEQ ID NO:179)

Based on MOTIF 9:

5' (G or A)AA(G or A)CANGC(A or G or T)AT(G or A)TCNA(G or A)(G or A)AA 3' (SEQ ID NO:180)

5' TT(T or C)(T or C)TNGA(T or C)AT(A or C or T)GCNTG(T or C)TT 3' (SEQ ID NO:181)

Based on MOTIF 10:

5' CCCAT(G or A)TC(T or C)(T or C)(T or G)NA(T or G or A)N(T or A)(G or A)(G or A)TC(A or G)TGCAT 3' (SEQ ID NO:182)

5' ATGCA(T or C)GA(T or C)(T or C)(T or A)N(A or C or T)TN(A or C)(A or G)(A or G)GA(T or C)ATGGG 3' (SEQ ID NO:183)

Based on MOTIF 11:

5' NA(G or A)N(G or C)(A or T)(T or C)T(T or C)NA(A or G)(C or T)TT 3' (SEQ ID NO:184)

5' (A or T)(G or C)NAA(A or G)(T or C)TN(A or G)A(A or G)(A or T)(G or C)N(T or C)T 3' (SEQ ID NO:185)

Based on MOTIF 12:

5' (A or G or T)(A or T)(A or T)(C or T)TCNA(G or A)N(G or C)(A or T)N(T or C)(G or T)NA(G or A)NCC 3' (SEQ ID NO:186)

5' GGN(T or C)TN(A or C)(G or A)N(A or T)(G or C)N(T or C)TNGA 3' (SEQ ID NO:187)

Replace the paragraph beginning on page 62, line 25, with the following paragraph.

Once produced, polyclonal or monoclonal antibodies are tested for specific *RSP* polypeptide recognition by Western blot or immunoprecipitation analysis (by methods described in Ausubel et al., supra). Antibodies which specifically recognize a *RPS* polypeptide are considered to be useful in the invention; such antibodies may be used, e.g., for screening recombinant expression libraries as described in Ausubel et al., supra. Exemplary peptides (derived from Rps2) for antibody production include:

LKFSYDNLESDLL (SEQ ID NO:188)

GVYGPGGVGKTTLMQS (SEQ ID NO:189)

GGLPLALITLGGAM (SEQ ID NO:190)